

An Emotional Behavior Study in Sprague-Dawley CD Rats Given  
Fluoxetine or Paroxetine Daily by Gavage from Postnatal Day  
(PND) 33 to 62

Study No. F3

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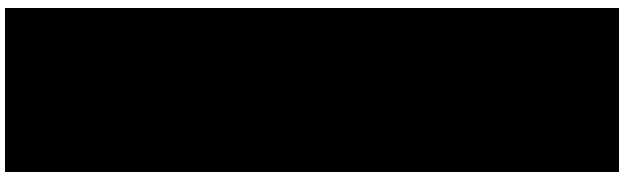
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## Signature Pages

Compound: Fluoxetine HCl (LY110140 HCl) Study: F3

The following individual is responsible for the design, conduct, and supervision of this study and for the data reported herein:

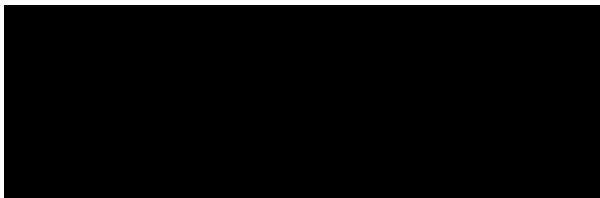


08/27/08  
Date

## Signature Pages (Continued)

Compound: Fluoxetine HCl (LY110140 HCl) Study: F3

The following personnel of Eli Lilly and Company have reviewed this report and agree with the interpretation of the data presented herein:



27 Aug 2008  
Date

## Summary

### An Emotional Behavior Study in Sprague-Dawley CD Rats Given Fluoxetine or Paroxetine Daily by Gavage from Postnatal Day (PND) 33 to 62

#### Report F3

de Jong and colleagues (2006) reported that chronic treatment of rats with the SSRIs fluvoxamine and paroxetine during adolescence resulted in changes in tests designed to measure emotional and sensory gating. The purpose of this study was to evaluate whether administration of fluoxetine HCl to rats during the same period affected behavioral performance on the same tests: anxiety-like behavior in the elevated plus-maze, depressive-like behavior in the forced swimming test, and sensory gating in the prepulse inhibition of acoustic startle test. Since paroxetine was shown to produce effects on these types of behaviors under similar conditions, it was included in this study as a comparator.

Fluoxetine HCl or paroxetine HCl was administered by oral gavage to two separate groups of rats from PND 33 to 62. Group A was tested for behavioral performance during the dosing period (initiated on PND 57), while Group B was tested approximately 2 months after dosing ceased (initiated on PND 122). Each group consisted of 20 male rats selected such that only 1 male per treatment was taken from any one litter (within-litter design). Control rats received purified water. Fluoxetine HCl doses were 3 and 10 mg/kg (expressed as the freebase and adjusted for purity). Paroxetine HCl doses were 3, 10, and 17 mg/kg (expressed as the freebase and adjusted for purity). The 17 mg/kg dose of paroxetine HCl was selected to match the 15 mg/kg paroxetine (base) dose given in the [de Jong study](#).

There were no effects on any of the behavioral parameters in any of the fluoxetine treated groups. Consistent with the de Jong study, the high dose paroxetine rats exhibited anxiogenic behavior in the elevated plus-maze. The number of head dips in the paroxetine 17-mg/kg group was significantly decreased, compared to controls, when evaluated during the dosing period (PND 57). Although not statistically significant, time spent in the open arm of the maze was also decreased in these rats at this age. There were no effects on any of the other behavioral endpoints in the paroxetine-treated rats. Behavior in the elevated plus-maze was not affected in the paroxetine-treated rats during the post-treatment evaluation (PND 122).

In summary, fluoxetine administration to rats during adolescence did not affect behavioral performance on 3 tests designed to measure emotional and sensory gating behavior.

## Study Elements/Methods

### Emotional Behavior Study

This study was not conducted in strict accordance with Good Laboratory Practice Standards (GLP).

[Appendix A](#) contains the study protocol.

|                                                   |                                                                                                                                                                       |
|---------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Study:                                            | F3                                                                                                                                                                    |
| Study initiation date:                            | First litter enrolled 9/29/07; first day of dosing 10/04/07                                                                                                           |
| Treatment period and frequency of administration: | Postnatal Day (PND) 33 to 62, daily                                                                                                                                   |
| Experimental dates:                               | 10/04/07-03/23/08                                                                                                                                                     |
| Test articles/compounds:                          | Fluoxetine HCl (LY110140 HCl)<br>Paroxetine HCl                                                                                                                       |
| Lot numbers/purity:                               | Fluoxetine HCl: A292718/98.5%<br>Paroxetine HCl: 78246-49-8/99%                                                                                                       |
| Vehicles:                                         | Purified water                                                                                                                                                        |
| Dose volume:                                      | 5 mL/kg                                                                                                                                                               |
| Route:                                            | Oral gavage                                                                                                                                                           |
| Species, strain, and supplier:                    | Rat, Sprague-Dawley CD/IGS,<br>Charles River Laboratories, Inc.<br>(Raleigh, NC)                                                                                      |
| Gender:                                           | Male                                                                                                                                                                  |
| Identification method:                            | Transponder code                                                                                                                                                      |
| Total number of animals on study:                 | 240 (n = 20/dose group/time point);<br>within litter design was used such<br>that an equal number of males from<br>each litter were represented in each<br>test group |

Treatment groups:

| Treatment Group | Dose (mg/kg)          |
|-----------------|-----------------------|
| 01              | 0 (dH <sub>2</sub> O) |
| 02              | 3 (fluoxetine HCl)    |
| 03              | 10 (fluoxetine HCl)   |
| 04              | 3 (paroxetine HCl)    |
| 05              | 10 (paroxetine HCl)   |
| 06              | 17 (paroxetine HCl)   |



#### Dose selection:

Fluoxetine doses were based on a previous study in juvenile rats in which rats received doses of 3, 10, and 30 mg/kg from PND 21 to PND 90 (██████████). The 30 mg/kg dose exceeded the maximum tolerated dose, based on severe decreases in body weight gain and clinical signs. Doses of 3 and 10 mg/kg were well tolerated and therefore were selected for this study. The dose level of 17 mg/kg of paroxetine HCl was selected to match the dose of 15 mg/kg of paroxetine base given in the de Jong study (2006). The 3 and 10 mg/kg doses of paroxetine were selected for a direct comparison to the doses used for fluoxetine.

#### Analysis of test articles:

Purity was reassayed from remaining test article following the treatment period to verify test article stability. Concentration verification samples were analyzed from one sample from each dose concentration from the 1<sup>st</sup> dose preparations.

#### Live-phase parameters:

Clinical signs (daily for mortality/morbidity); body weights (weekly except during dosing treatment period which was daily)

#### Behavior:

##### *Elevated plus-maze:*

Group A rats tested on PND 57; Group B rats tested on PND 122; black plastic maze consisting of 2 open arms (10 x 50 cm) and 2 closed arms (10 x 50 x 50 cm) radiating from a center platform and elevated 50 cm above floor level; each rat was placed on the center platform facing an open arm; duration of test equaled 5 minutes; behavior of rats was digitally recorded and quantified directly from video by measuring time spent in open arms, counting the number of entries into the open arms, measuring the latency to first enter the open arms, and counting the number of head dips.

##### *Prepulse inhibition of startle response:*

Group A rats tested on PND 59; Group B rats tested on PND 124; performed in acoustic startle chambers of San Diego instruments; chamber contains Plexiglas tube resting on plastic frame; piezoelectric accelerometer mounted under tube which detects and transduces motion of the tube; stimulus delivery done using SR-LAB software, via a speaker mounted 10 cm above the cylinder; computer software also digitized, rectified, and recorded response of accelerometer; with 100 ms readings collected beginning at stimulus onset; startle amplitude defined as average of 100 readings; throughout session, background noise of 70 dB was maintained; after 5-minute habituation, ten blocks of five trials was delivered to measure prepulse inhibition; each block consisted of one startle trial (120 dB, 20 ms broad band burst), one no-stimulus condition, and three different

prepulse/startle pairs administered pseudo randomly; in the pairings, the prepulse was approximately 3, 5, or 10 dB above background; prepulses were broadband burst followed by the 120 dB startle pulse 70 ms later; interval between two trials varied between 10 and 20 s; startle amplitude was calculated as mean of 10 trials; degree of prepulse inhibition (in percentage) was calculated as  $(1 - \text{average startle amplitude on prepulse trial} / \text{startle amplitude on startle trial}) \times 100$ .

*Forced swimming test:*

Group A rats tested on PND 61 and PND 62; Group B rats tested on PND 126 and PND 127; test consisted of a training session of 15 min and a test session of 5 min, 24 hr later; for both the training and test sessions, each rat was placed in an acrylic cylinder (25 cm high and 20 cm diameter) filled with water at 20°C up to 22 cm (rat could not reach floor without diving); behavior was observed by trained individual and digitally recorded and quantified directly from the video by measuring time spent trying to climb out or swimming around versus staying immobile.

Corticosterone:

Animals were removed from the FST and taken immediately to another room, decapitated and blood samples collected. Blood was collected in polypropylene tubes containing EDTA, centrifuged at 4°C for 15 min, plasma aliquoted, and stored at -80°C until assayed for corticosterone. For the assessment of corticosterone, plasma was diluted 1:10 in assay buffer and assayed in duplicate using a commercially available EIA kit for corticosterone (IDS, Fountain Hills, AZ).

Storage and location of live-phase data:

Neuroscience Research Laboratories,  
Division of Neurology, Cincinnati  
Children's Research Foundation,  
Cincinnati, OH

Storage and location of bioanalytical data:

Frozen blood samples were stored in  
a -80°C freezer located on R5 of the  
Research Foundation exclusively  
used by [REDACTED].

## Statistical Evaluation

Data were collected by computer or were video recorded on DVD and later scored by experienced technicians according to criteria described above in [Methods](#). The resulting data were entered into Excel spreadsheets and crosschecked for accuracy. Checked data were transferred to [REDACTED] for statistical analyses. She checked the data against the master list of subjects enrolled in the experiment. She also checked the data against the variance records to ensure that all irregularities (if any) were attended to. She also checked the entered data for outliers (none were identified in this project). Data were then analyzed by mixed linear model analyses of variance using SAS ProcMixed procedure (SAS Institute, Cary, NC). In cases where there was a repeated measure factor, best fit statistics were used to select the covariance matrix model that best fit the data. This reliably turned out to be the Autoregressive (AR(1)) model. Degrees of Freedom were calculated using the Kenward-Roger method. The data for Group A and Group B animals were analyzed separately. Acoustic startle data were analyzed by prepulse intensity, hence the analysis was a group x prepulse ANOVA. In addition, the prepulse trials were separately analyzed with the zero-prepulse trials used as a covariate to determine if there was a prepulse effect independent of baseline startle response intensity. An analysis of covariance (ANCOVA) was used for this evaluation. The results of FST, EPM, and corticosterone were analyzed by simple 1-way ANOVA for group. Body weights were analyzed using 2-factor group x week ANOVAs with week as a repeated measure factor. A posteriori group comparisons were performed using the method of Hochberg.

## Results and Conclusions

### Test Articles

Test solutions of both compounds were prepared and sent to ABC Laboratories for analytical chemistry in order to determine of drug concentrations by HPLC analysis (Methods; M-1736-000 and USP 30 Paroxetine HCl). Each sample was analyzed in triplicate.

### Concentration Verification:

A first set of samples was taken from the prepared test solutions for concentration verification. Mean actual concentrations of the test solutions were within 3% of target concentrations.

| Test Article | Target concentration (mg/mL) | Actual concentration (Mean, mg/mL) | % of Target |
|--------------|------------------------------|------------------------------------|-------------|
| Paroxetine   | 0.6                          | 0.588                              | 98          |
|              | 2.0                          | 2.05                               | 103         |
|              | 3.41                         | 3.30                               | 97          |
| Fluoxetine   | 0                            | not detected                       | -           |
|              | 0.6                          | 0.60                               | 100         |
|              | 2.0                          | 1.98                               | 99          |

### Stability Assessment:

Fluoxetine hydrochloride is know to be stable in solution  $\geq 7$  days. Stability was determined for paroxetine at the low and high concentrations at three time points: immediately after preparation and at 3 and 7 days. The paroxetine solutions were stable over the 7-day period.

| Target concentration (mg/mL) | Time point (days) | Actual concentration (Mean, mg/mL) |
|------------------------------|-------------------|------------------------------------|
| 0.6                          | 0                 | 0.581                              |
|                              | 3                 | 0.637                              |
|                              | 7                 | 0.634                              |
| 3.4                          | 0                 | 3.37                               |
|                              | 3                 | 3.54                               |
|                              | 7                 | 3.58                               |

### End of Study Drug Purity:

After the completion of the experiment, crystalline samples of both drugs were sent to ABC Laboratories to determine drug purity. Both paroxetine HCl and fluoxetine HCl were of high purity (mean percent potency of 101%).

### Body Weights

Body weights were only minimally decreased in the 10-mg/kg fluoxetine rats and the 17-mg/kg paroxetine rats (Figures 1-3). Decreases in body weights generally occurred during the second week of dosing through the end of the dosing period. However, by the second week of the post-treatment period, body weights of all treatment groups were comparable to controls, demonstrating complete reversibility (Figure 4).

### Elevated plus-maze

Treatment with fluoxetine did not affect performance in the elevated plus-maze. All parameters evaluated were similar to control values. The 17-mg/kg paroxetine rats demonstrated significantly reduced numbers of head dips (Figure 6) and, although not statistically significant, less time in the open arms of the maze (Figure 5) when tested during the dosing period. This anxiogenic behavior is consistent with the findings reported by de Jong and colleagues (2006) suggesting that this finding is reproducible in this laboratory under these conditions. Latency to enter the open arms was unaffected during the dosing period (Figure 7). There were no effects in the paroxetine treated rats when tested during the post-treatment period.

### Prepulse inhibition of startle response

Treatment with fluoxetine or paroxetine did not affect prepulse inhibition of the startle response (Figures 8 and 9).

### Forced swimming test

Treatment with fluoxetine or paroxetine did not affect time spent immobile in the forced swimming test (Figure 10).

### Corticosterone

Treatment with fluoxetine or paroxetine did not affect corticosterone levels (Figure 11).

### Overall conclusion

Fluoxetine administration to rats during adolescence did not affect behavioral performance on 3 tests thought to measure emotional behavior. However, consistent with the de Jong study, paroxetine administration to rats during adolescence resulted in anxiogenic behavior as demonstrated in the elevated plus maze.

## References

██████████. A study in young CD (IGS) rats administered fluoxetine hydrochloride (LY110140) orally by gavage to evaluate general, reproductive and behavioral toxicity. WIL Research Laboratories, Inc., Ashland, OH.

de Jong TR, LJA E Snaphaan, T Pattij, JG Veening, MD Waldinger, AR Cools, B Olivier. 2006. Effects of chronic treatment with fluvoxamine and paroxetine during adolescence on serotonin-related behavior in adult male rats. *Eur Neuropsychopharm* 16, 39-48.

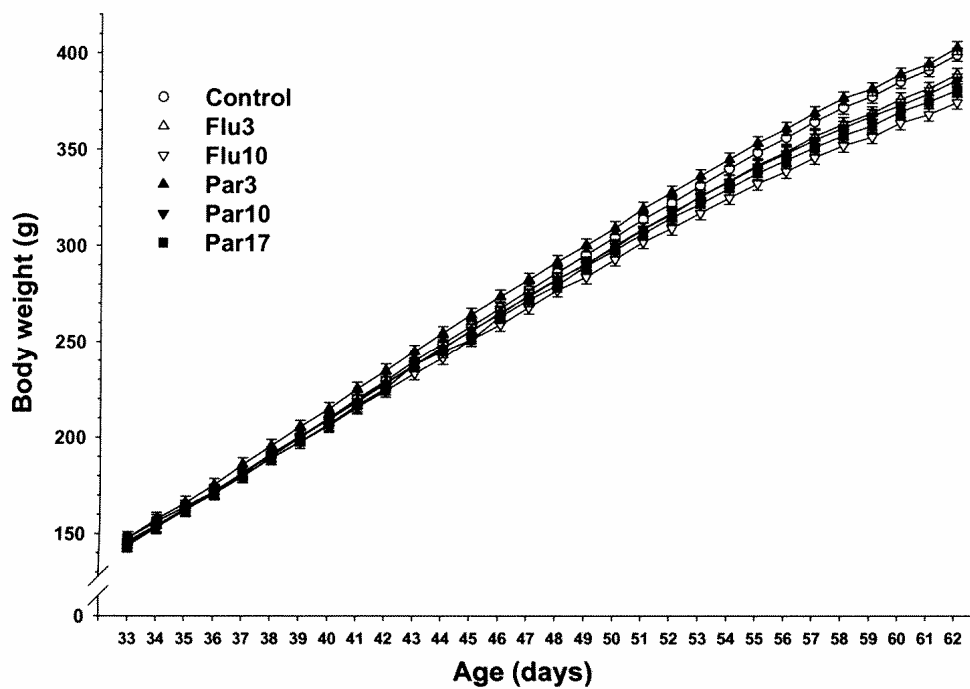


Figure 1: Body weight of male rats throughout the dosing period.

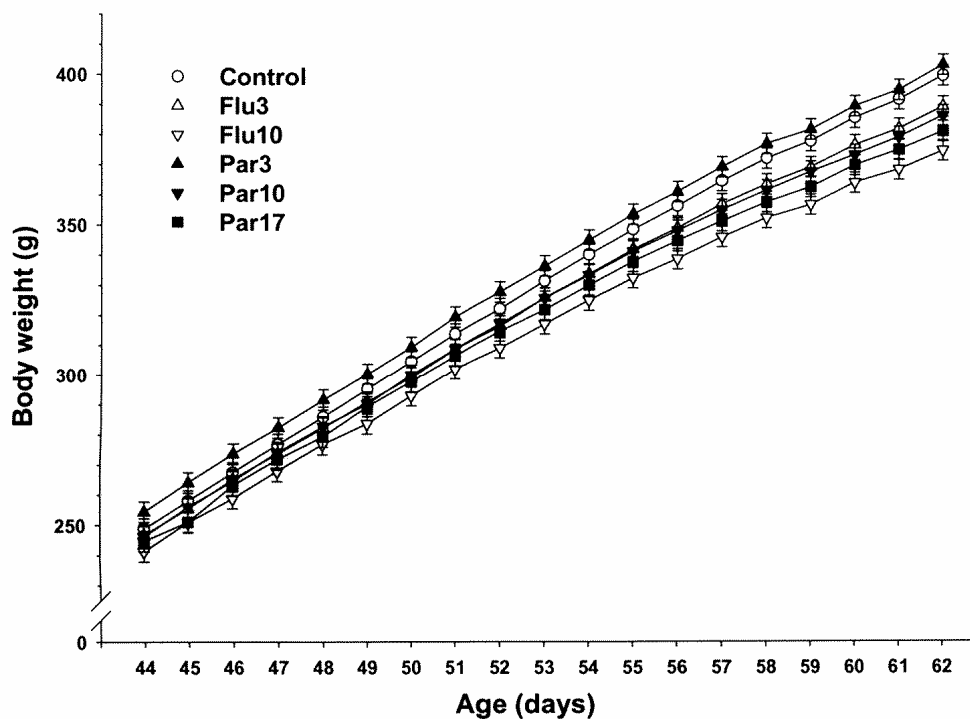


Figure 2: Days of age on which there were significant body weight differences (inclusive) (ANOVA,  $p \leq 0.05$ ).



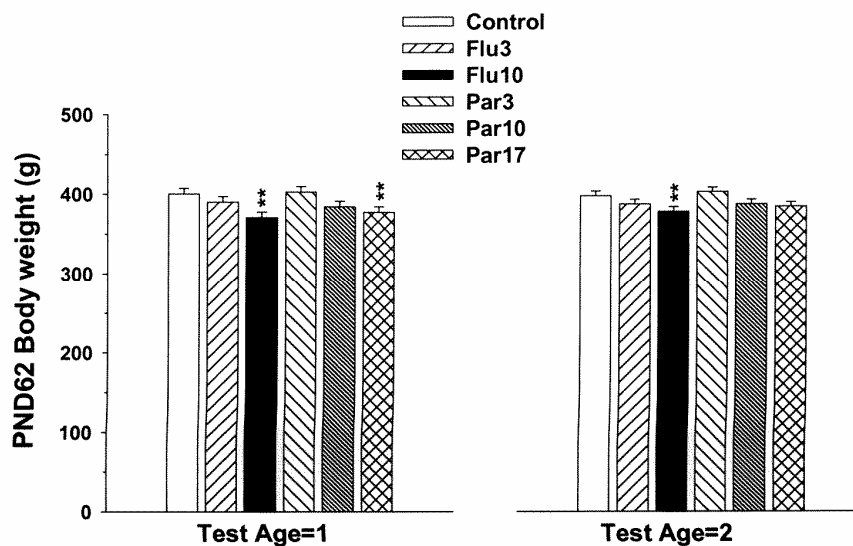


Figure 3: Body weight for Group A (tested during the dosing period) and Group B (tested approximately 2 months post-treatment) shown on the last day of treatment (PND 62). Since Groups A had completed testing and Group B had not yet begun testing, they are shown separately

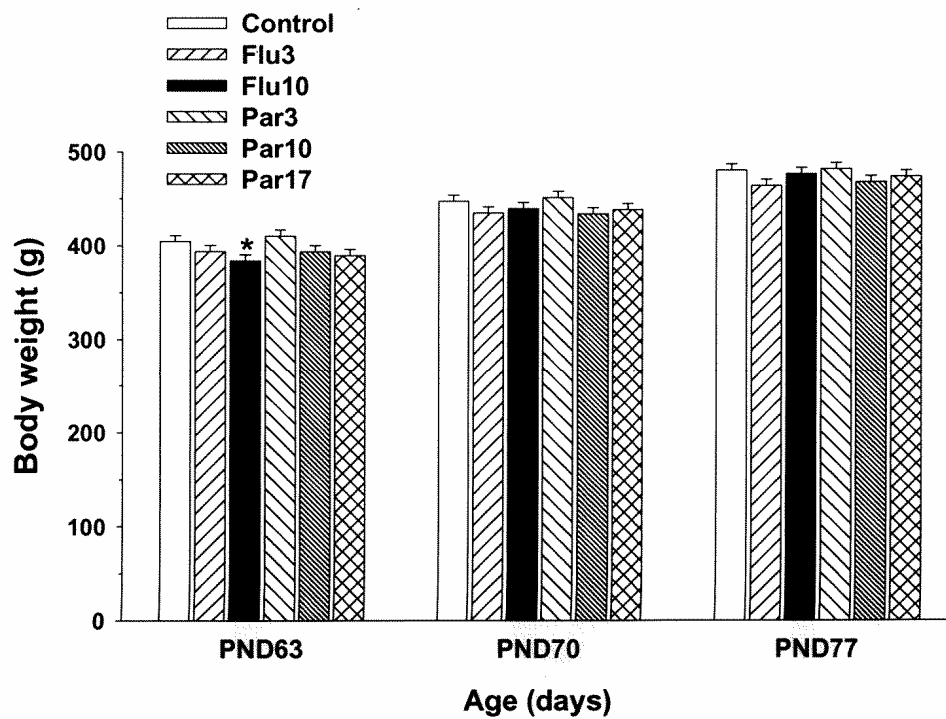


Figure 4: Body weight of male rats (Group B) shown weekly for the weeks after the end of treatment in order to illustrate body weight recovery.

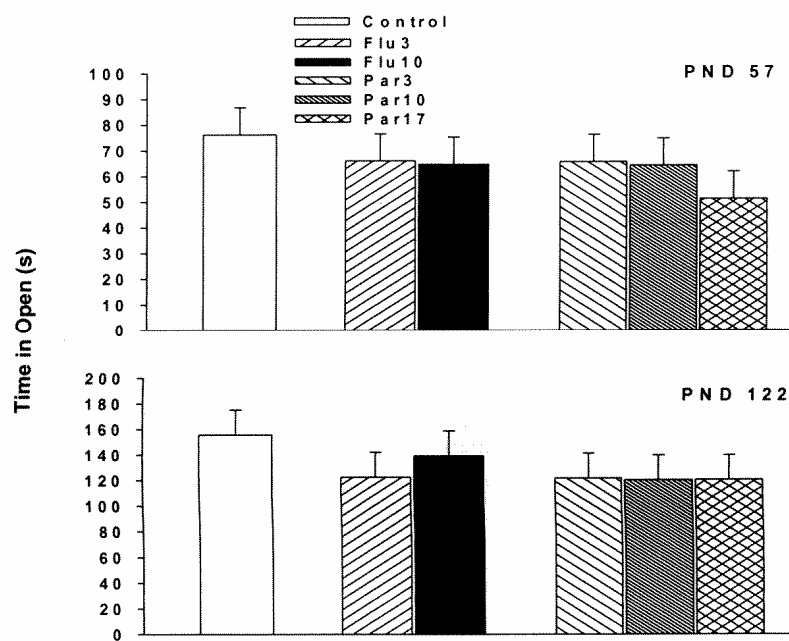


Figure 5: Time spent in open arms of elevated plus-maze

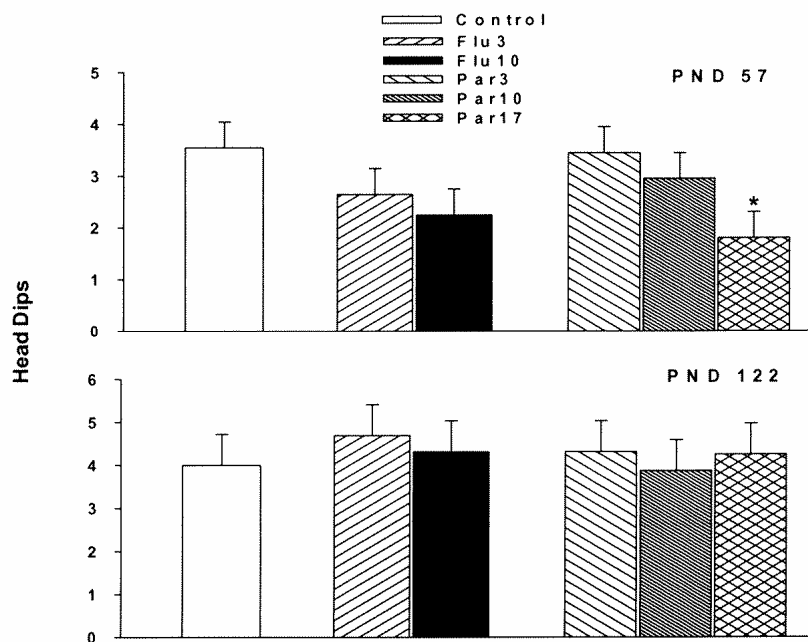


Figure 6: Number of head dips in elevated plus-maze.

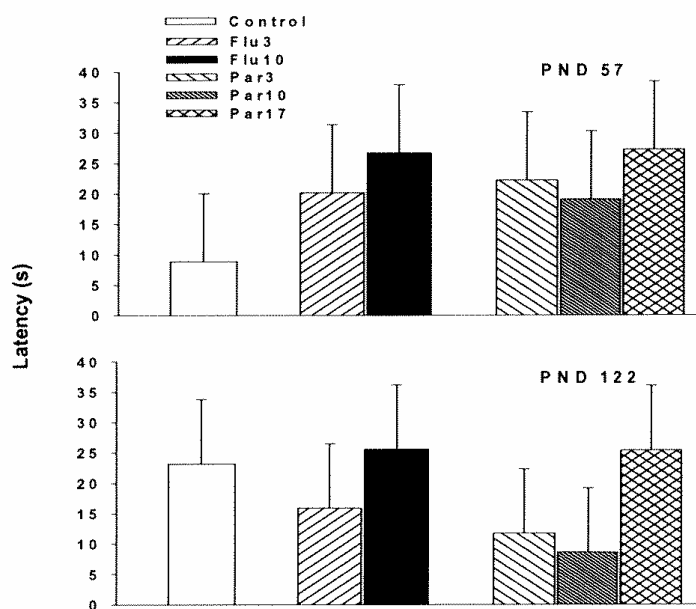


Figure 7: Latency to enter first open area in the elevated plus-maze.

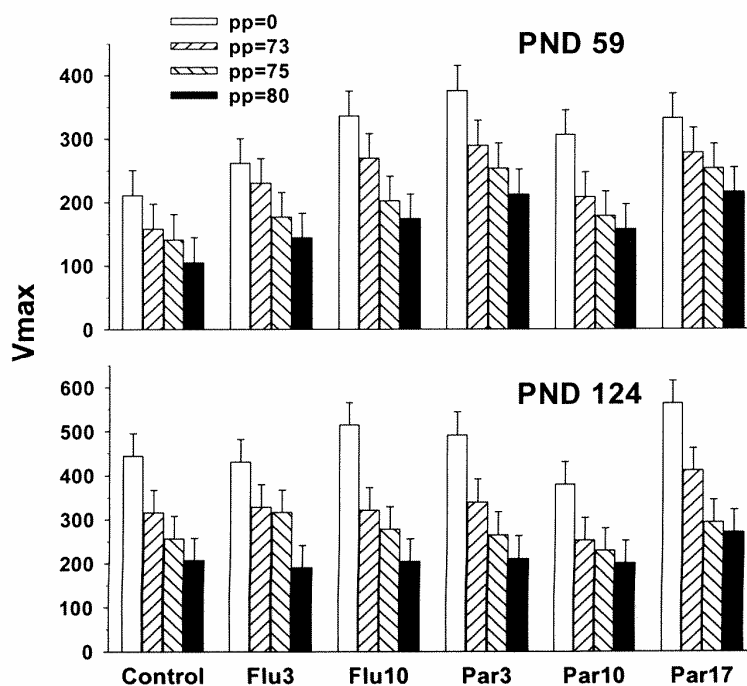


Figure 8: Acoustic startle/Prepulse inhibition test. Vmax is the maximum startle amplitude measured during the first 100 ms after stimulus onset (measured in arbitrary units of mV). pp = prepulse intensity in db (SPL). The startle stimulus was 115 dB.

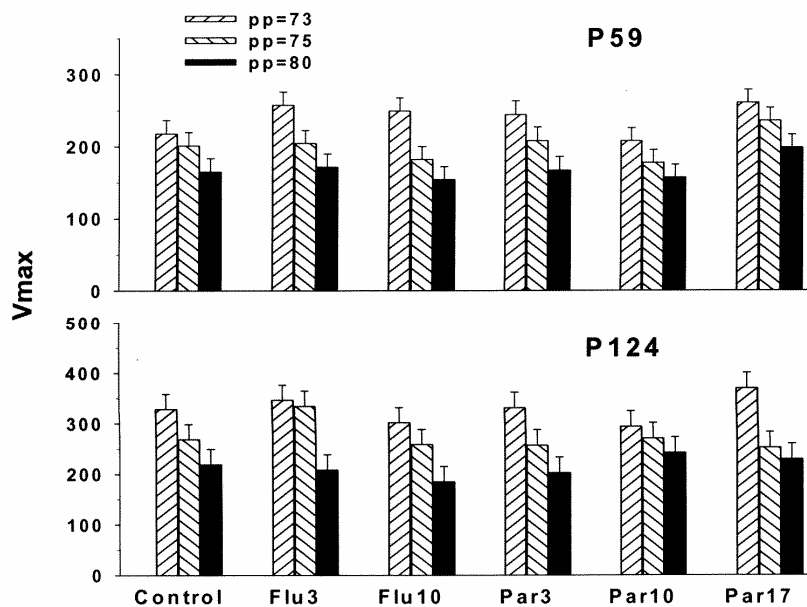


Figure 9: Acoustic startle/Prepulse inhibition pp0 (no prepulse) trials used as a covariate in an ANCOVA analysis.

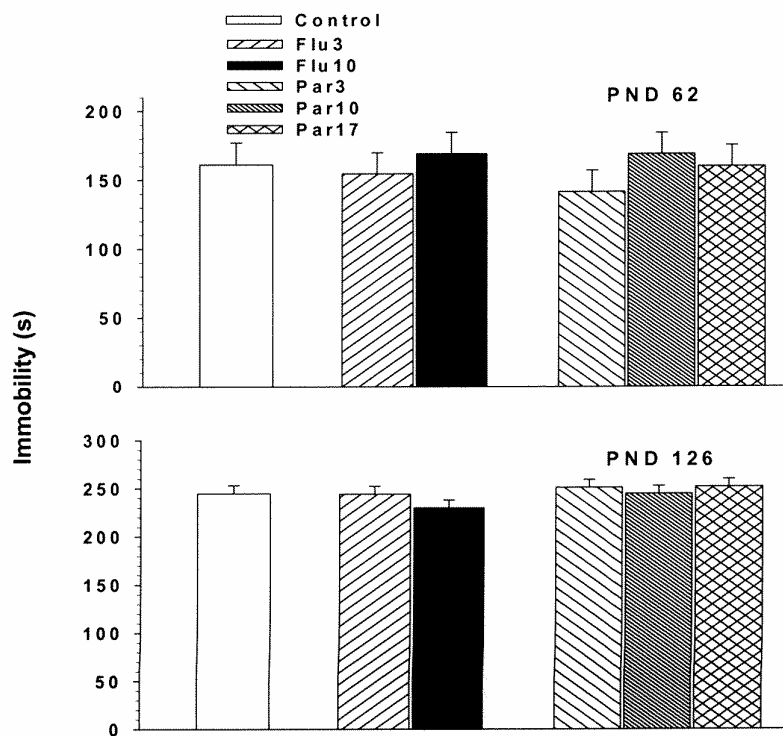


Figure 10: Time spent immobile in test session (5 minute total duration) of forced swimming test.



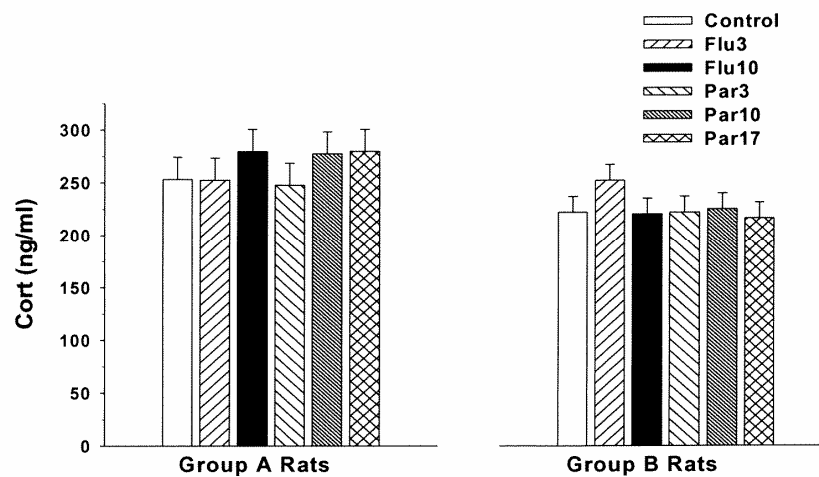


Figure 11: Corticosterone levels in plasma

## Appendix A

### Protocol F3

Protocol # 5D03015

10/5/2007

Amended 2-6-08

**Hypothesis:** Fluoxetine will not produce similar effects on anxiety-related behaviors as paroxetine following administration of either drug during the adolescent period (P33-62).

### Purpose:

The purpose of this study is to evaluate whether administration of fluoxetine HCl (LY110140\_hydrochloride) or paroxetine HCl by gavage to juvenile rats from postnatal day (P) 33 to 62 affects behavior in the elevated plus-maze, the startle/prepulse inhibition test, and/or the forced swim test (Porsolt).

### Breeding and Delivery

Male (251-275g) and nulliparous female (151-175g) Sprague-Dawley CD IGS rats will be obtained from Charles River Laboratories (Raleigh, NC). Rats will be maintained on a diet of NIH-07 feed. Rats will acclimate to the animal room for at least one week prior to breeding. Embryonic day 0 (E0) will be the day a sperm plug is detected. Females will be removed from breeding within 2 days of E0. Starting on E21, pregnant females will be checked twice daily for the presence of a litter, once in the morning and once in the late afternoon. Birth will be termed P0. Only males will be used, therefore litters will be culled to 8 pups (6 males and 2 females) on P1, although only 5 males will be treated. **No cross-fostering will be used to complete litters.** Offspring will be ear punched on P10. There will be a total of 40 litters. Twenty litters will be tested during drug administration starting on P57 and 20 litters will be tested 60 d after drug administration starting on P122. The within litter factor will be treatment; therefore all treatments will be represented in a litter.

### Treatments and Drug Administration

Fluoxetine HCl (LY110140 hydrochloride expressed as the freebase and adjusted for purity) or paroxetine HCl (expressed as the freebase and adjusted for purity) or the distilled water vehicle (CON: water will be sterile, USP) will be given by gavage (5 ml/kg). Drugs will be mixed fresh daily at first. Pups will be assigned to dose groups using a random assignment system (random number table) with the restriction that the study is a within litter design so that all treatments are represented within a litter. Males in each litter will receive one of the following:

- (1) USP water
- (2) Fluoxetine 3 mg/kg
- (3) Fluoxetine 10 mg/kg

- (4) Paroxetine 3 mg/kg
- (5) Paroxetine 10 mg/kg
- (6) Paroxetine 17.07 mg/kg

Whole litters will be divided into 2 different test-age groups, those that begin on P57 and those that begin on P122. Dosing will be from P33-62 and animals will be weighed prior to each dose. All dosing will be after behavioral testing for the day and occur between 1200-1600 h each day. Animals that appear to be affected by the administration of drug will be noted on the dosing sheets.

### **Body weights, weaning, and housing:**

Nulliparous females are bred in-house with proven sires. Birth is designated as P0. Animals are weighed on P1 and culled to 6 males and 2 females. Animals will be naturally weaned by the dams and separated into pairs on P28. All litters will receive stainless steel huts in their cages as part of our standard practice as provided by Veterinary Services. Huts will be placed in the cages when pregnancy is confirmed and will remain in the cages of the dams prenatally, postnatally (during lactation), and in the cages of the offspring throughout the experiment. Pups are weighed weekly until dosing begins at which time the animals are weighed daily prior to each dose. After the dosing period animals will again be weighed weekly. After P28, animal will be re-housed in same-sex pairs in box cages for the remainder of the experiment. Females will be euthanized at weaning and only males retained. On P28, all males will be implanted with an IPTT-300 (BMDS) for identification purposes. Animals will be lightly anesthetized with isoflurane and injected with the transponder.

### **Behavioral Testing**

**Group A: animals begin testing on P57**

**Group B: animals begin testing on P122**

### **Elevated plus-maze**

- Begins on the appropriate day, either P57 or P122
- The black plastic maze consists of two open arms (10 x 50 cm) and two closed arms (10 x 50 x 50 cm) radiating from a center platform (10 x 10 cm) and is elevated 50 cm above floor level.
- Animal ID# will be made visible to the video camera at the start of each animal's test by being written on a white-board with erasable marker and placed on the floor facing up under the apparatus.
- The DVR must be started FIRST before the trial begins and the test animal placed in the plus-maze in the center of the maze facing an open arm. The experimenter will immediately leave the test room.
- Animals will be tested for 5 minutes with only a single halogen light (room lights off) at the preset low-level marked on the lamp's rheostat. The window in the door will be blocked.
- Feces and urine will be removed between tests and the runway cleaned with 70 % ethanol between animals.

- Test sessions are digitally recorded and behavior scored later for time in open areas, time in closed areas, head dip frequency, and number of open area entries. An animal will be considered in the open area when it has 2 paws beyond the central border and its head out in the open.

#### **Acoustic Startle/PPI (measures sensorimotor gating, hearing, and reflex function)**

- Begins on either P59 or P124
- Acoustic startle reactivity will be measured in an SR apparatus (obtained 3-2007 from San Diego Instruments, San Diego, CA)
- Rats are placed in an acrylic cylindrical test chamber that is mounted on a platform with a piezoelectric force transducer attached to the underside of the platform. The platform is located inside a sound-attenuated test chamber.
- Throughout the startle session, a background noise of 70 dB will be maintained (set by SDI software).
- When the test session begins, the software includes a 5 min acclimation period preceding test trials.
- The test chamber fan switch should be placed in the “off” position.
- After the habituation period, ten blocks of five trials will be delivered to measure prepulse inhibition.
- Each animal will receive a 5 x 5 Latin square sequence of trials that are of 5 types: no stimulus (NS), startle trial with no prepulse (SS), prepulse trial with prepulse set at 3 dB above background (i.e., 73 dB; PPI-3), prepulse trial with prepulse set at 5 dB above background (i.e., 75 dB; PPI-5), or prepulse trial with prepulse set at 10 dB above background (i.e., 80 dB; PPI-10). Hence, the Latin square will present each trial type in each order once (NS, SS, PPI-3, PPI-5 and PPI-10). The Latin square will be repeated twice without interruption which will provide 50 trials as per deJong et al. (2006).
- Trials of the same type are averaged together.
- The intertrial interval varies randomly within the test session from 10-20 sec as set using the SDI software.
- The startle signal is 20 ms 120 dB SPL mixed frequency burst (white noise). The startle recording window is 100 ms. Stimuli are set using the dB(C) scale on the Quest sound level meter. The sound level meter is calibrated with a Quest standardized sound calibrator. Prepulse duration is 20 ms. Hence in the SDI software the set-up is PP on for 20 ms, then an 80 ms ‘wait’ interval, then startle signal on for 20 ms. The recording window is 100 ms from startle signal onset.
- The ISI (interval from prepulse onset to startle signal onset) = 100 ms as per deJong et al. (2006).
- The degree of prepulse inhibition (in percentage) will be calculated as (1-startle amplitude on prepulse trial/ startle amplitude on startle trial) x 100.
- Following the test for each animal, the apparatus will be cleaned with 70% ethanol.

#### **Forced Swim**

- Begins on P61 ends on P62 or begins on P126 ends on P127.

- The test will consist of a training session of 15 min and a test session of 5 min, 24 hr later.
- A transparent acrylic cylinder (25 cm high and 20 cm diameter) will be filled 2/3 full with water at a temperature of 21-23°C.
- On day 1, animals will be placed in the water for 15 min and digitally recorded.
- On day 2, animals will be replaced in the water for 5 min and digitally recorded.
- Immobility time will be scored. Immobility is defined as no motion except what is required for the animal to stay afloat. Time to immobility will also be recorded.
- After every session the rat will be dried with a towel and the cylinder will be emptied and refilled with fresh water.

### **Sacrifice and plasma collection**

Immediately following the second day of forced swim, the animal will be brought to an adjacent suite and decapitated. Trunk blood will be collected. The blood will be centrifuged for 25 minutes at 1,300 rcf and the plasma will be removed and assayed for corticosterone levels.

### **Data Analysis**

All data recorded by computer are backed-up each day using a back-up routine to the hard drive of the local computer and also transferred to one of the CCRF servers for mass data storage and labeled with the study name and test (e.g., F3, startle). [REDACTED] [REDACTED] downloads these files to her computer's hard drive and backs these files up on her hard drive. Forced swim data are to be recorded using a DVR on DVDs for later scoring manually (and entered into an Excel file). Elevated Plus-maze data are also recorded by DVR on DVDs and scored manually by [REDACTED]. Scored data are then transferred to [REDACTED] computer. All daily logs made by technicians during testing are also transferred to [REDACTED]. [REDACTED] checks all data against the study design, list of animals enrolled in the study, and against the daily logs for conformity and checks for any irregularities. Discrepancies (if any) are resolved between [REDACTED] and the technicians prior to data analysis. Means and standard errors are calculated by group for each test and control groups reviewed for similarity to historic controls.

Inferential statistical testing for treatment-related effects will be performed on all data using SAS programs.

Data are subjected to statistical analysis using factorial analysis of variance models using SAS programs (Statistical Analysis System; SAS Institute, Cary, NC, version 9.1.3). For startle, the model at each test age will be a group x trial-type ANOVA, blocked by litter, in which group is a between-subject factor and trial-type is a repeated-measure factor and the model will be Proc Mixed (mixed linear model). Best fit statistics will be used to select the variance-covariance model with optimal fit to the data. Treatment group main effects will be followed up with Bonferroni step-down pairwise group comparisons. Group x trial-type significant interactions will be further analyzed using the slice ANOVA option within Proc Mixed and those slices that are significant further analyzed for group differences using the Bonferroni step-down method. Forced swim and Plus-maze will be analyzed by simple one-way ANOVA (which only requires Proc GLM). If the group effect is significant, follow up group comparisons will be made

using the Bonferroni step-down method. Body weights will be analyzed by group x age ANOVAs with age as a repeated measure factor. Data for each test at the two different test ages will be analyzed separately.

#### Plasma Corticosterone

When each animal completes FST, it will be immediately taken to another room, decapitated and trunk blood collected. Trunk blood will be collected in ice-chilled tubes containing 0.05 ml EDTA. Blood will be centrifuged for 25 min at 4 °C, plasma collected, and stored at – 80 °C until assayed for CORT. Hormone titers will be determined using ELISA kits (IDS, Fountain Hills, AZ ).

#### Key Personnel

